

Engineering of enzymatic reactions and reactors for lipid modification and synthesis

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1 Introduction

Enzymatic lipid modification and synthesis have been dramatically developed in the last 20 years from a simple laboratory idea at the beginning to an industrial reality at present. This development has produced countless scientific publications worldwide through various channels. A simple literature search through useful databases such as ISI and Food Science and Technology Abstract can give more than 3000 of related publications in the area of lipids. This development has also generated a variety of potential products in every part of lipid processing and applications. This in turn has attracted wide attention from our related industry sectors. A strong push from this trend to the industrial strategies for future development has been quietly under way. More and more companies have organized their efforts to exploit the possibility using enzyme technology for their future commercial business. So far more than ten international companies have used enzyme technology for their product development and industrial production. A few products are also in commercial markets such as human milk fat substitutes (HMFS), cocoa butter equivalents (CBE), confectionary anti-bloom agents, diacylglycerol (DAG) cooking oil, polyunsaturated fatty acid concentrates, etc.

These interesting progresses are largely attributed to the development of micro-aqueous enzymology. Traditionally, enzymes of such kind (hydrolases) are supposed to work in water-abundant systems for hydrolysis, as demonstrated in nature. Under these conditions, hydrolysis is the only reaction that can be applied for limited applications in lipid areas. Although lipases for lipid hydrolysis have been known for decades before the discovery of possible uses of enzymes in micro-aqueous systems, none of the industrial applications had really been used in practice. In 1984, Zaks and Klivanov published a paper in *Science*, indicating that enzymes could work in a solvent micro-aqueous medium at 100 °C [1]. This is an important milestone in enzyme history. It means that enzymes can also be used for the catalysis of reverse reaction, i.e. esterifi-

cation. This opens up a variety of potential applications. In the lipid area, a reaction such as interesterification (acydolysis, alcoholysis, and ester-ester exchange) is also possible with the catalysis of lipases in a probable hydrolysis-esterification two-step mechanism. Thus chemical interesterification, which has been implemented in the current industry can be fully replaced.

However, the current situation is the joint result of many progresses. A simple summary of these progresses can be given in the following list:

- understanding of the enzyme 3D structure based on protein engineering,
- understanding of interfacial activation of lipases,
- use of genetic engineering in enzyme production,
- improvement of lipase immobilization technology,
- understanding of water thermodynamics in microaqueous enzymatic reactions,
- use of “solvent” engineering for certain reactions,
- development of new interesting products and the increase of their market demands,
- development of more efficient enzyme processes.

It is certain that more items can be added to this list by different people in different areas of research. In general, the joint effort of all these developments provides us with the possibility, flexibility, versatility, and economical potentiality to be applied in commercial sectors and industrial exploitation. The driving forces for the involvement of industrial sectors can be also listed as follows:

- very interesting products and potential market demands,
- improvement of product quality refereeing to mild conditions,
- reduction of enzyme price and balanced economy in general,
- simple processes and low investment,
- environmental considerations,
- customer perception and marketing strategy,
- know-how preparation and technology takeover/control.

This list can be prolonged: “image” strategy by using enzyme technology is important for many industrial companies; energy-efficiency of the technology is also often mentioned. A healthy industrialization of the technology can be reflected by the economical turnover and overall performance of the commercial sectors. The latter is often more complicated than the technology itself.

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* This paper is dedicated to Professor Carl-Erik Høy, Biochemistry and Nutrition, BioCentrum-DTU, who was one of my close colleagues and friends. He died on February 8, 2003.

There are many updated books, journal reviews, and book chapters that have been dedicated to the progresses in the area. A few selected references, purely from the author's preference, are provided for further reading [2–7]. In this paper, an overview of the enzymatic reactions and reactors for the modification and synthesis is provided. The major enzymatic reactions for the modification of lipids are often referring to hydrolysis, esterification, acidolysis, alcoholysis, and ester-ester exchange from the reaction mechanisms. Interesterification or transesterification is also commonly used in literature to describe some of the above reactions. A detail description is given below for the following two terms:

- **Interesterification:** a general term for the reactions between an ester and a fatty acid, an alcohol, or another ester, which includes acidolysis, alcoholysis, and ester-ester exchange. In literature, it sometimes describes only ester-ester exchange or alkaline-catalyzed randomization of oils and fats.
- **Transesterification:** a term often used for ester-ester exchange. It is also used in literature for the acidolysis reaction or as a general term for all the three reactions including alcoholysis.

Particular concern is given to some selected examples to illustrate the progresses in the area. The engineering aspects of reactions and reactors lie in the stable and practical reaction system, acceleration of the reaction equilibrium, shifting of reaction equilibrium towards products through process design, reaction and reactor optimization through modeling of related factors, and others. Some of these points will be discussed in this paper.

2 Enzymatic hydrolysis

There are three major routes currently used for the hydrolysis of fats and oils in the production of fatty acids: high pressure steam splitting, alkaline and enzymatic hydrolysis. The high temperatures and pressures (typically 250 °C, 7×10^6 Pa needed for steam splitting make this process unsuitable for splitting sensitive triacylglycerols (TAGs). There are also difficulties associated with alkaline hydrolysis, namely high energy costs and the need to acidify the soaps formed. Thus enzymatic processes are imperative alternatives in certain circumstances.

Lipases are known in nature for hydrolysis. This reaction catalyzed by lipases has been well known for more than five decades. There had been some applications in early stages for the purpose of producing free fatty acids in certain industrial sectors. However, this application had not really attracted much interest, probably due to the high cost of the lipases, limited product potentialities, and complex reaction systems.

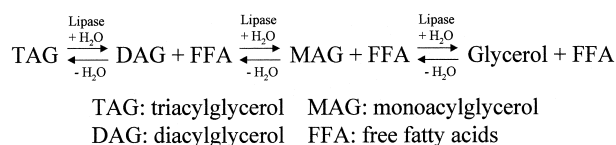


Fig. 1. Steps of enzymatic hydrolysis of oils and fats.

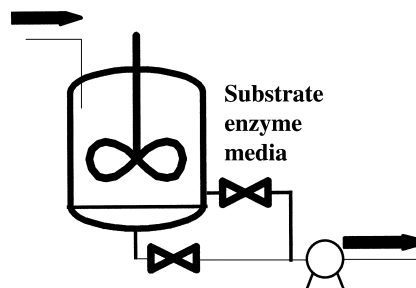


Fig. 2. A simplified illustration of a batch-stirred tank reactor.

This situation has been changed much in the last 10–20 years due to the overall progresses in the area. Enzymatic hydrolysis of oils and fats involves three steps from TAGs to glycerol and free fatty acids (FFA). The reaction is described in Fig. 1.

As shown in the figure the reaction is a multi-phase system consisting of at least the oil phase and the water phase. In most cases, a liquid enzyme is used which is dissolved in the water phase. A batch-stirred tank reactor (STR) is often used for the reaction (Fig. 2). Enzyme recovery for long-term reuse is not possible since the latter will be in the same phase with water and glycerol, even though circulation of the water phase can be used in certain applications. In some cases, a solvent system is used in order to obtain a practical system, especially with immobilized lipases. A few factors affecting the reaction equilibrium and efficiency are listed Tab. 1.

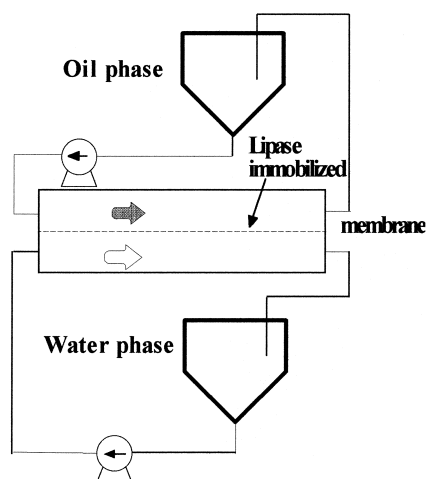
Packed bed reactors (PBR) are another type of reactors that can be used for the hydrolysis of oils and fats through lipase catalysis. Solvent media might help to maintain the stable system. In general this is not very practicable, especially in large scales. The large amount of water and the production of glycerol will easily clog the packed bed and make it impossible to operate. However, for low-degree hydrolysis, the operation might be still feasible with limited amounts of water in the system, as in the application described in the last paragraph of this section.

One of the interesting systems for the enzymatic hydrolysis of oils and fats is the application of a membrane reac-

Tab. 1. A description of factors that influence the reaction equilibrium and efficiency of enzymatic hydrolysis.

Factors	Remarks
Lipase	Lipase dispersion is the main concern to affect the reaction efficiency. Lipase water solution can be dispersed in a few different ways: – Mechanistic stirring: this is often difficult in large-scale operation for a strong stirring. In a small-scale operation, reaction rates were increased with the increase of stirring rates to 1000 rpm (unpublished results). Strong stirring enforces the contact between oils and water/lipase phase. – Electrostatic dispersion: negatively charged lipase solution is sprayed into the oil phase. This operation provides very small droplets into the oil phase and the contact surface is increased. The dispersion is much more stable due to the negative charges. – Emulsion system with surfactants: surfactant is added in the beginning to increase the contact between oil and water/lipase.
Water	The increase of water content can shift the reaction to hydrolysis as shown in Fig. 1. On the other hand, too much water also dilutes the lipase and makes the system more difficult. 5–50 wt-% water has been used in various publications. In case solvent is used, the supply of water is often conducted by the maintenance of water activity in the system.
FFA	FFA are formed during the reaction. They are inhibitive to the reaction. In certain cases, FFA can be continuously removed from the system by certain manipulation such as membrane separation, soap formation, etc.
Glycerol	Glycerol is also the direct product from the reaction. Glycerol exists in the water phase. This can be separated through water phase circulation or membrane separation.

tor [8–9]. In the typical example, the lipase is immobilized on the surface of the membrane (Fig. 3). The oil phase is circulated in the lipase section and the water phase is circulated on the other side. Reactions occur on the membrane surface and the formed glycerol will be transported through the membrane to the water phase. The FFA in the oil phase can be separated by a separate membrane system or by distillation and the acylglycerols are re-circulated for further hydrolysis. The glycerol in the water phase

**Fig. 3.** A membrane system for the enzymatic hydrolysis of oils and fats for the production of free fatty acids.

can be also further treated before being circulated back by a third membrane system or another purification step. In theory this is a perfect set-up. However, much effort has to be made before the actual application is feasible. The membrane might be the hurdle to develop this process into potential production.

One of the interesting applications of hydrolysis is the enrichment of polyunsaturated fatty acids (PUFA) from fish oil [10–11]. Some lipases have less activity towards very long chain fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Partial hydrolysis of fish oil can largely release those shorter chain fatty acids such as 12, 14, 16, and 18 carbon fatty acids. Therefore the content of PUFA in the acylglycerols can be dramatically increased. A typical operation in a 1-kg scale could produce an acylglycerol product with more than 50% PUFA from a fish oil with 23% PUFA (D. Zhou, X. Xu, unpublished observations). A time course of the reaction is given in Fig. 4.

3 Enzymatic esterification

Esterification is the reverse reaction of hydrolysis. This reaction is only possible and useful in a microaqueous reaction system where hydrolysis can be minimized and controlled with limited amounts of water in the system. In water-abundant reaction systems, hydrolysis is the main reaction and it is difficult to make a useful reaction for esterification. A large number of applications has been

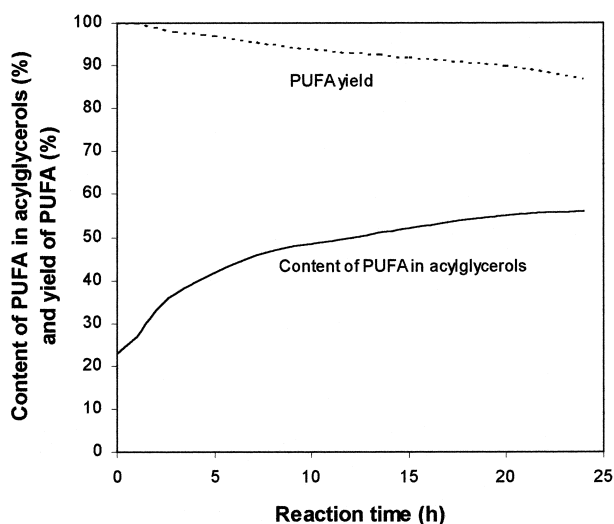


Fig. 4. Time course of the polyunsaturated fatty acids (PUFA, EPA+DHA) in acylglycerols and PUFA yields for the Lipozyme RM IM-catalyzed hydrolysis of fish oil in a 1-kg level batch stirred tank reactor. Reaction conditions: 6 wt-% lipase, 45 °C, water/oil 0.6/1 (wt/wt), stirring 500 rpm, and 100 ppm Toco 50.

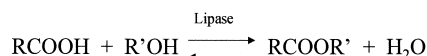


Fig. 5. A simple description of enzymatic esterification.

studied on the basis of extensive studies of microaqueous enzymology. This gives a variety of possibilities to exploit the enzyme technology for ester synthesis.

Esterification is the simple reaction between an organic acid and an alcohol. The reaction is depicted in Fig. 5. Water is one of the direct products from the reaction and it has dramatic effects on the shifting of reaction equilibrium. It has to be continuously removed from the reaction system in order to minimize the reverse hydrolysis reaction. The common methods to remove water from the reaction system in laboratory experiments or industrial applications are listed in Tab. 2.

Water in the reaction system is in one way the reaction by-product, which should be removed in order to force the reaction to the product side. On the other hand, a certain dynamic water environment should be maintained in or-

Tab. 2. Possible methods to remove water from the reaction system in laboratory experiments or industrial applications during the enzymatic esterification.

Vacuum evaporation	The system is simply applied with vacuum. The optimal vacuum should be used to maintain a certain water activity in the reaction system. A vacuum too high may lead to low enzyme activity due to the non-optimal water activity in the reaction system.
Membrane pervaporation	A membrane pervaporation system with vacuum is applied to remove the water from the reaction system. A suitable water activity in the reaction system can be maintained by an automatic retro-control system with a humidity sensor.
Azeotropic solvent distillation (external drying agents)	The azeotrope property of alcohol or other solvent medium can be used to remove water from the reaction system. A distillation system is applied and the distilled solvent or alcohol can be dried in a separate connected chamber with a drying agent. After drying the solvent alcohol can be fed back to the reaction system.
Drying agent (internal or external)	Drying agents such as a molecular sieve or silica gel are added to the reaction system in certain stages of the reaction to remove water. It is very useful for large-scale plant operations. In a solvent system, non-contact drying can also be used by placing the drying agents in the vapor.
Air or N ₂ bubble	Bubbling by dry air or nitrogen can be also used to remove water, particularly in small-scale experiments.
Alcohol partition	Water will be largely kept in the alcohol phase in the partition between the hydrophilic alcohol phase and the hydrophobic fatty acids phase. By using a large amount of alcohol phase, the water in the system can be kept away from the reaction interface.
Solvent engineering	In certain laboratory operations, a hydrophilic solvent may be used to extract water and keep water away from the reaction.
Salt pair (internal)	In laboratory experiments, salt pairs with certain water activity can be used to maintain the water activity of the system.
Water activity control (external)	Vapor phases in the reaction system are circulated with the vapor phase of the saturated salt solution or salt pair. Water content or activity in the reaction system can be thus regulated.

der to have a high enzyme activity. This is especially important for the long-term production in an industrial plant. The evacuation rate of water from the reaction system should be carefully controlled in order not to dry out the whole system. In certain developments, automatic control of water humidity is a useful solution with a humidity sensor in the reaction system and a retro-control system to the vacuum pump.

A higher lipase activity usually requires a certain water activity (a_w) that often locates in the higher a_w range. The product yield, however, requires an a_w as low as possible in practical experiments. For this reason, the first stage of reaction can be controlled at a higher level of a_w to maintain a higher reaction rate. For the last stage of reaction, however, lower a_w can be used in order to improve the product yields. This strategy has been used in a large number of studies [12–13]. The reaction efficiency and yield have been indeed improved.

In Fig. 5, a number of different organic acids and a large range of alcohols can be used for the synthesis of a variety of products. Some selected examples are listed in Tab. 3. In most of these applications, the reactions involve three phases in the simple reaction system in a batch STR: a hydrophobic phase, a hydrophilic phase, and a solid enzyme phase (Fig. 6). Particular concern should be paid to ensure an efficient reaction system including water removal, homogeneity, and system sustainability. In many cases, solvents have to be used as the medium to increase the homogeneity depending on the particular application. Solid-phase biocatalysis has also been used for the reaction system in cases like sugar esters and other carbohydrate esters [14].

Packed bed reactors have been applied in previous work for the esterification to produce esters [15]. Due to the difficulty to remove water from the system, a circulation sys-

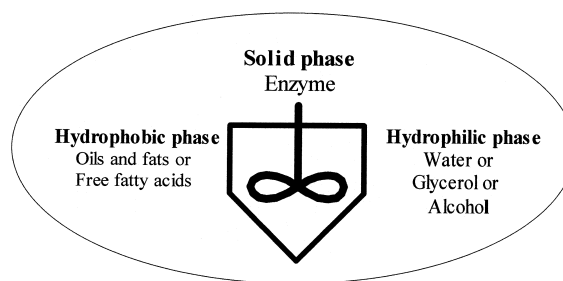


Fig. 6. An illustration of the three-phases reaction system.

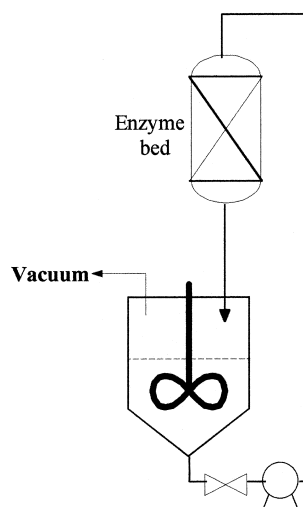


Fig. 7. A circulation system with a packed bed reactor unit and a storage tank with vacuum applied for esterification to produce esters.

tem is often used, in which a packed enzyme bed is the reaction unit (Fig. 7). Vacuum is applied to the circulation tank to remove water. In a practical process, stepwise operation instead of circulation can be implemented in which a packed bed together with a tank is a unit step. The num-

Tab. 3. Typical acids and alcohols for the enzymatic esterification together with a list of useful ester products that have been studied.

-COOH providers	-OH providers	Products
Fatty acids (C1–C26)	Straight or branched chain alcohols (C1–C28)	Biodiesels, fatty acid esters, wax
Other acids:	Glycerol (DAG, MAG)	MAG, DAG, TAG
Ferulic acid	Glycols	Glycol esters
Sinapic acid	Geraniol (citronellol)	Terpene esters and short chain esters
Caffeic acid	Sterols	Sterol esters
Anthranilic acid	Flavonoids	Flavonoid esters
Quinic acid	Lutein	Lutein esters
Glucuronic acid	Ascorbic acid	Ascorbyl esters
Kojic acid	Glucose	Sugar esters
Others	Lyso-phospholipids	Phospholipids
	Pyridoxine	Sunscreens
	Others	Others

ber of steps can be designed depending on the reaction efficiency in each step and the final product quality. An on-line water removal along the packed enzyme bed, in principle, can be also designed with an internal layer penetrative only for water vapor or with a tube in order to allow continuous vacuum. Other technical solutions to improve the feasibility of easy and practical operation, process efficiency and stability, etc. can be expected.

Membrane reactors have been widely used in the esterification system for different purposes [16]. As in the hydrolysis system for the production of FFA, the two-phases system can be also used for esterification applications (Fig. 3). Lipase can be immobilized on the membrane surface and products formed will be either in the hydrophobic phase or the hydrophilic phase depending on the product property. In most cases, products are likely to be in the hydrophobic phase such as acylglycerols formed from the reaction between glycerol and FFA.

Membrane has been also used for the removal of water through water pervaporation [17]. This is better implemented in large-scale or practical processes. Water can be more efficiently removed through the membrane matrix and oil phase will not be affected. A process diagram is provided to illustrate the process (Fig. 8).

4 Enzymatic acidolysis

Acidolysis has been more widely explored for the modification of lipids using enzyme technology. Chemical acidolysis has fewer applications in reality due to no positional specificity. The progress of enzyme technology makes acidolysis an important key word. The reaction takes place between an ester and an acid. The acid will

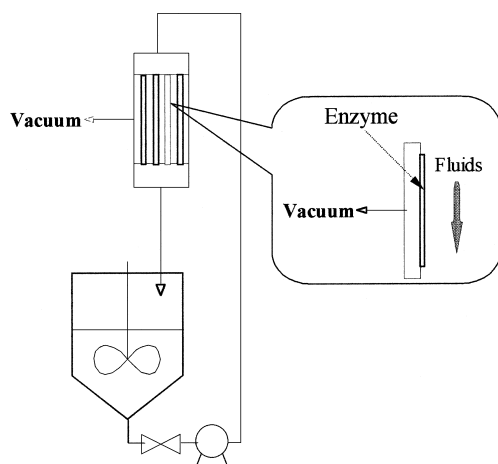
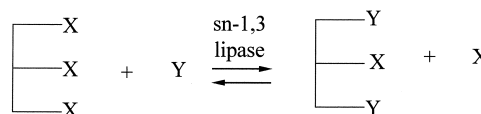


Fig. 8. A membrane reactor system together with pervaporation and circulation through a storage tank for esterification to produce esters.



X and Y are two types of fatty acids.

Fig. 9. A principle illustration of enzymatic acidolysis between a TAG (XXX) and a fatty acid (Y).

be exchanged with the acid in the ester. The reaction is depicted between a triacylglycerol and a FFA with a *sn*-1,3 specific lipase (Fig. 9).

In principle the reaction can be conducted between any organic esters and acids. In most applications in the lipid field, esters can be tri-(di-, or mono-)acylglycerols, glycerophospholipids, alkyl fatty acid esters, etc. and acids can be fatty acids or other acids as listed in Tab. 3 in the esterification section.

Enzymatic acidolysis reaction is a reversible reaction. The reaction is commonly considered as a two-step reaction: hydrolysis and esterification. For this consideration, diacylglycerols are considered as the reaction intermediates. Through steps of hydrolysis and esterification, the new fatty acids in the system are incorporated into triacylglycerols. The reaction will finally reach equilibrium. This procedure for the reaction between a TAG (LLL) and a fatty acid (M) is depicted in Fig. 10.

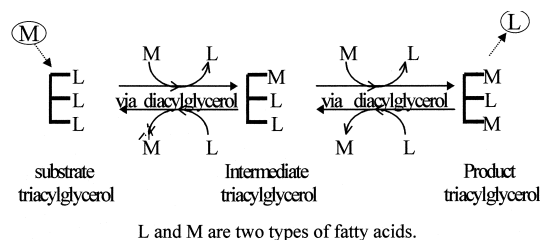


Fig. 10. Reaction illustration of enzymatic acidolysis between LLL and M with *sn*-1,3 specific lipases.

Diacylglycerols (DAGs) are also the precursor of side reactions since they are not thermodynamically stable [18]. The fatty acids will migrate to the neighbor position in the glycerol backbone. This leads to a positional non-specificity of the lipases and the formation of by-products. The whole reaction between LLL and M is depicted in Fig. 11. There are relationships between DAGs and product and byproduct yields. The relationship is illustrated in a reaction between a linoleic acid-based oil and capric acid for the production of C10:0/C18:2/C10:0 (Fig. 12).

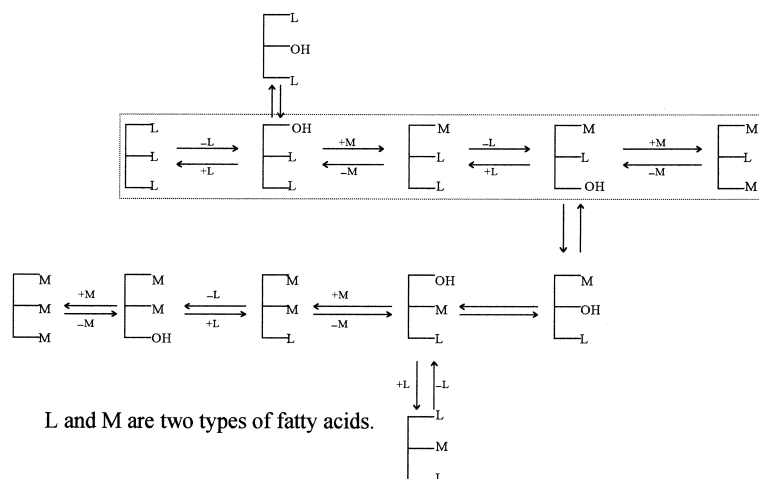


Fig. 11. A step-by-step reaction pathway for the main reactions and side reactions during the enzymatic acidolysis between LLL and M. *Sn*-1,3 specific lipases are used.

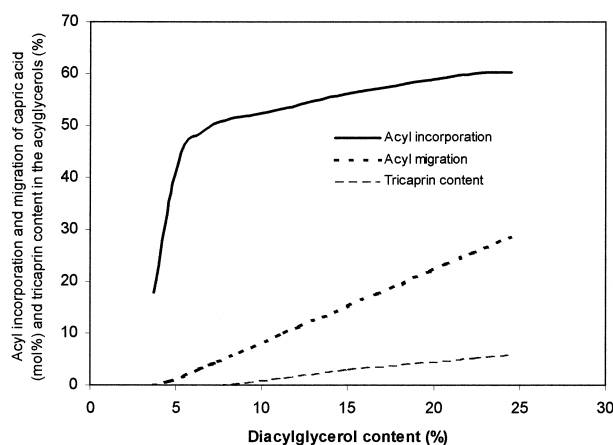


Fig. 12. Relationship between diacylglycerol content and acyl incorporation, acyl migration, and tricaprinn content in the acidolysis reaction between sunflower oil and capric acid for the production of structured lipids. Reaction conditions: Lipozyme RM IM 10 wt-%, temperature 60 °C, sunflower oil/capric acid 1:6 (mol/mol), and water content 7 wt-% based on enzyme. This shows that diacylglycerols are both intermediates for the main reactions and also side reactions.

Enzymatic acidolysis is a reversible reaction and the equilibrium will be eventually reached. The product yield under reaction equilibrium is decided by the substrate ratio, *i.e.* the ratio between fatty acids and esters [19]. For the reaction between TAGs and FFA, the incorporated fatty acids in the product can be calculated as shown in Tab. 4. However, the rate to reach equilibrium is related to many parameters such as reaction temperature, enzyme dosage, water content, and reaction system. If inhibition and other factors exist, the time to reach equilibrium will also be longer.

To shift the reaction equilibrium to the product side as shown in Fig. 10, we can try to move the products out of

the reaction system. As for structured TAGs, the main product may not be easy to be separated from the system since its property is very similar to the starting oil. However, the released FFA can be removed in some cases [20–21]: for example in the reaction between tricaprillin and DHA for the purpose to produce DHA/C8:0/DHA, the caprylic acid is released. Caprylic acid has a much smaller size than DHA. This difference can be used for the removal of caprylic acid from the system by an internal membrane system. More than 15% additional incorporation beyond the reaction equilibrium has been obtained by this operation. In the case of tri-PUFA production by the reaction between tri-C4:0 and PUFA, the released butyric acid (C4:0) can be removed continually out of the reaction

Tab. 4. Theoretical content of incorporated fatty acids at different substrate ratios and reaction types provided that the reaction equilibrium is reached.

Ratio between FFA and TAG [mol/mol]	Content of incorporated fatty acids [mol-%]	
	<i>sn</i> -1,3 specific reactions (no migration)	Non-specific reactions
1	22.2	25.0
2	33.3	40.0
3	40.0	50.0
4	44.4	57.1
5	47.6	62.5
6	50.0	66.7
7	51.8	70.0
8	53.3	72.7
9	54.5	75.0
10	55.6	76.9
Maximum content [†]	66.7	100.0

[†] Maximum content of incorporated fatty acids under the assumed structure of the products.

system by simple vacuum. This leads to a yield of tri-PUFA of up to 95% compared to very small yields without vacuum application since butyric acid is strongly inhibitive for many lipases. This has been an alternatively good method for the synthesis of tri-PUFA instead of esterification method.

The reaction has been widely used for the production of structured TAGs such as cocoa butter substitutes (SOS; S = saturated fatty acids, O = oleic acid), anti-bloom agent (BOB; B = behenic acid), structured lipids (MLM; M = medium-chain fatty acids, L = long-chain polyunsaturated fatty acids), human milk fat substitutes (UPU; U = unsaturated fatty acids, P = palmitic acid), etc. The reactions are obviously catalyzed by *sn*-1,3 specific lipases since the positional specificity is essential for the final product.

The system for this reaction is considerably simple compared to esterification since both material oils and acyl donors are hydrophobic and in the same phase for most applications. This makes the process setup simple and easy. Both batch STRs and PBRs have been widely used for this reaction system from laboratory small scales to industrial plants. Since positional specificity is essential for this reaction in most applications, the reaction time should be as short as possible since acyl migration during the reaction process is proportional to the reaction time. For this consideration, PBRs have been considered the best reactors for this reaction system [22–23]. Reaction in a PBR has shown to generate very low acyl migration compared to the reaction in a STR (Fig. 13). A simple pilot plant setup of the PBR is given in Fig. 14.

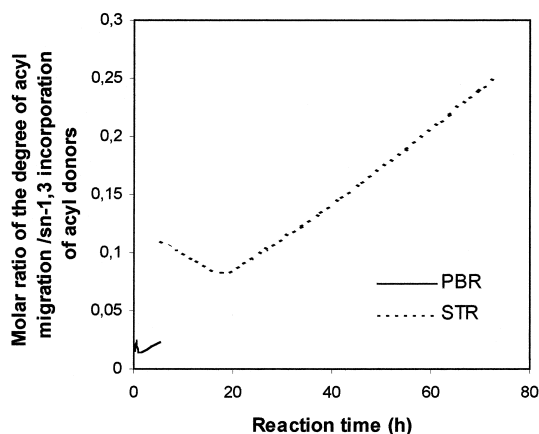


Fig. 13. A comparison between packed bed reactor (PBR) and stirred tank reactor (STR) with respect to the extent of acyl migration formed per unit of *sn*-1,3 incorporation during the time course of the acidolysis reaction between rapeseed oil and capric acid with the catalysis of Lipozyme RM IM. Very low acyl migration occurs in PBR because of the much shorter reaction time.

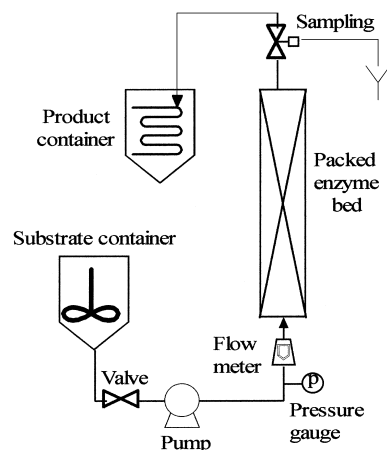


Fig. 14. A simple description of packed bed reactor.

5 Enzymatic alcoholysis

Alcoholysis is the reaction between an ester and an alcohol. Chemical alcoholysis has been widely used in industry for the production of partial acylglycerols, such as MAGs and DAGs, and biodiesels such as fatty acid methyl esters or ethyl esters. In the case of the former the reaction between oils and fats and glycerol (glycerolysis) is used, whereas the reaction between oils and fats and methanol or ethanol (methanolysis or ethanolysis, respectively) is exploited for the latter. Enzymatic alcoholysis has been widely studied for the production of similar products. The benefits of using enzyme processes vary from case to case but the general advantages of enzyme technology can be applied as described in the introduction section. Some particular applications can be highlighted as follows:

- *sn*-2 MAGs, the intermediate for the synthesis of structured TAGs, can be produced with *sn*-1,3 specific lipases.
- MAGs containing PUFA of higher quality can be produced, since milder conditions are applied than in chemical processes.
- Easy recovery of glycerol and possible uses of waste oils and fats are a partial argument for the enzymatic production of biodiesels.

Alcoholysis is also a reversible reaction as shown in Fig. 15. The starting ester (Ester¹) can be acylglycerols, TAGs or alkyl esters for example. The starting alcohol (Alcohol¹) can be glycerol, methanol, ethanol, or sterol for example. The product esters (Ester²) can be partial acylglycerols,

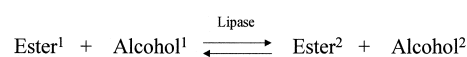


Fig. 15. An illustration enzymatic alcoholysis.

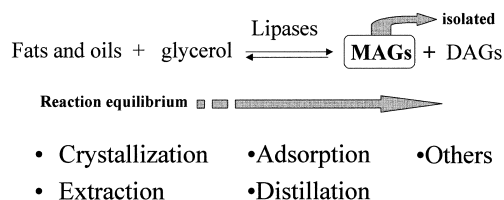


Fig. 16. Scheme and methods to drive the enzymatic glycerolysis of oils and fats towards the formation of monoacylglycerols (MAGs). DAGs – diacylglycerols.

alkyl fatty acid esters, or sterol esters and product alcohol (Alcohol²) can be glycerol or other alcohols.

To drive the reaction to the direction of the product side, we can use the different properties of the starting materials, which commonly exist for most applications [24–25]. For the reaction of glycerolysis, the product ester and alcohol are both MAGs and DAGs. In order to force the reaction towards more MAGs for example, we can isolate the formed MAGs from the reaction system by many different ways (Fig. 16). A brief description of the techniques is summarized in Tab. 5.

For the reaction of methanolysis or ethanolysis of oils and fats, the main products are the fatty acid methyl esters or ethyl esters and glycerol will be the by-product. Glycerol can be separated with the help of a membrane interface

through nano-filtration by applying pressure, or membrane extraction by applying methanol or ethanol as the extraction phase on the other side of the membrane. The formed glycerol will be transported to the alcohol side. The alcohol phase will also supply necessary alcohol for the proceeding of the reaction in the oil and fat phase.

Another way to drive the reaction to the product side is the use of special chemical agents, vinyl ester for example. For the production of MAGs, the reaction between vinyl fatty acid ester and glycerol can be conducted. The reaction should produce MAG and vinyl alcohol. The formed vinyl alcohol will quickly change to acetaldehyde. The latter is volatile even at low temperature and can be easily removed. This makes the alcoholysis reaction irreversible and directly leads to the formation of MAGs.

Glycerolysis of oils and fats has been widely used for the production of partial acylglycerols since 1 mol TAG and 2 mol glycerol can, in principle, form 3 mol MAG, with no unwanted byproducts. The content of partial acylglycerols is reached after reaction equilibrium and can be theoretically calculated from the ratio between glycerol and TAGs, provided the reaction is fully non-specific. The progress of the acylglycerols profile with the increase of the substrate molar ratio is described in Fig. 17. It shows that maximum 44% MAGs can be obtained at reaction equilibrium under the ratio of 2. With the increase of the ratio, the yield of MAGs is not increased if glycerol is included. Usually glycerol can be

Tab. 5. A description of techniques to isolate monoacylglycerols (MAGs) from the enzymatic glycerolysis of oils and fats system.

Methods	Brief description
Crystallization	MAGs have different melting points from TAGs and DAGs. The difference is shown in Tab. 6 for typical fatty acids. From this difference, the system can be controlled in a lower temperature after a certain period of reaction at higher temperatures. Under the lower temperature, MAGs will be solidified and this will drive the reaction to the formation of MAGs. Higher yield of MAGs up to 90% has been reported. Since the solidification, the system cannot be stirred at the lower temperature and the reaction is actually slow. Reaction time can be a few days. Every oil or fat has a suitable temperature to achieve this effect. This temperature is called critical temperature (T _c). Usually the lipase cannot be reused since it is difficult to separate it from the system.
Extraction	Particular solvents have different solubility to MAGs from oils and fats, DAGs, and glycerol. By this difference, the reaction can be driven to the formation of MAGs. In many cases, the system is actually no need to apply a separation stage during the reaction. The solubility difference makes MAGs away from the reaction interface.
Adsorption	In a two-phase operation system especially with a membrane between the oils and fats phase and glycerol phase (as shown in Fig. 3), the formed MAGs can be continuously removed by an external adsorption column implemented in the oil phase circulation line and the TAGs and DAGs left will be re-circulated back to the system for further reaction.
Distillation	Distillation is a separate operation to remove MAGs. The residues can be fed back for further reaction. This in general also drives the reaction toward MAG formation. Usually molecular or short path distillation can be used.

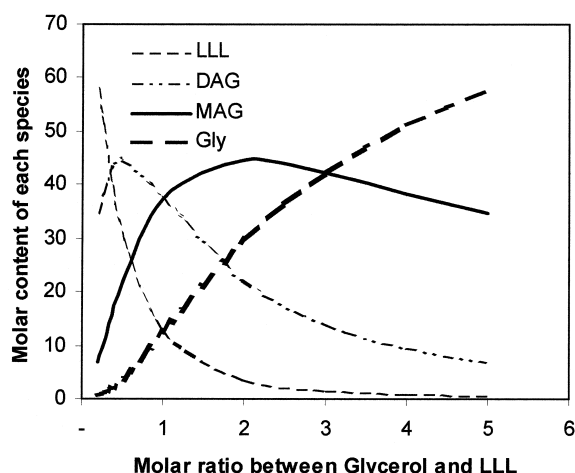


Fig. 17. The relationships between substrate molar ratio (glycerol/LLL) and the contents of acylglycerol species at equilibrium during reactions catalyzed by nonspecific lipases, which are assumed to have the same preference to the three positions. LLL, triacylglycerol; DAG, diacylglycerol; MAG, monoacylglycerol; Gly, glycerol.

easily removed and is not present in the final products. The content of MAGs can thus be increased and the amount of leftover TAGs can be further reduced.

Besides the production of partial acylglycerols and alkyl esters, alcoholysis can be used as part of the reaction steps for the production of structured TAGs. Ethanolysis is often used in this case for the first step of reaction to produce *sn*-2 MAGs with *sn*-1,3 specific lipase. The formed *sn*-2 MAGs can thus be used for the esterification with a particular fatty acid to form ABA-type structured TAGs [27, 28]. With different process set-ups, the formed *sn*-2 MAGs can be separated through crystallization before further esterification is conducted, or the reaction can be driven to form high purity MAGs in the system using particular lipases and reaction systems in which no purification steps are necessary before the esterification is proceeded. This process can lead to structured TAGs of high purity compared to a single-acidolysis process.

Alcoholysis is usually a three-phase system as described in Fig. 6. STRs are usually used since it is more difficult to

make a homogeneous system, in particular when no solvents are used. For the glycerolysis of oils and fats, glycerol is the difficult part, limiting the reaction rate. Glycerol usually binds to the lipase surface and makes the reaction impossible since oils and fats are very difficult to diffuse through this hydrophilic medium. The reaction rate is thus very low in a simple mixing system. Many ideas have been explored to increase the reaction rate [24–25]. Some of these ideas can in one way improve the reaction efficiency by improving the distribution of glycerol in the system as listed below:

- absorption of glycerol in silica gel,
- micelle formation by adding dioctyl sulfosuccinate in a solvent system,
- micro-emulsion system,
- use of solvents such as methyl-tert-butyl ether,
- use of supercritical fluids to form the system,
- two-phase system with a membrane interface.

Unless a stable single-phase system can be built, PBRs are not practical for the alcoholysis reaction since the reaction needs two phases in the stable fluid. For methanolysis or ethanolysis of oils and fats, a stable system using a lower alcohol ratio can be easily created at higher temperatures. Since alcohol at a high ratio can lead to lipase inhibition, stepwise addition of alcohol is imperative [29]. Therefore it is not a big problem to apply the continuous operation with PBRs. However, pressure drop of the PBR will be high and the system will need special care to ensure a stable system. For the glycerolysis reaction of oils and fats, solvent(s) has/have to be used to obtain a stable system in order to use a continuous process with PBRs. With the use of particular solvents, this can be achieved without big problems.

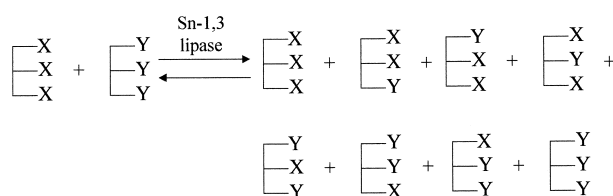
6 Enzymatic ester-ester exchange

Ester-ester exchange is a reaction between two esters. Acids will be exchanged between the hydroxyl moieties. Chemical ester-ester exchange, often referring to as chemical interesterification, is dominantly used for the randomization of oils and fats, which has been widely applied in industry for oil and fat modification. Enzymatic ester-ester exchange is an alternative to the chemical

Tab. 6. Difference of melting points of different acylglycerols with different fatty acids[†].

Fatty acids	FFA	<i>sn</i> -2 MAG	<i>sn</i> -1(3) MAG	<i>sn</i> -1,2 (2,3) DAG	<i>sn</i> -1,3 DAG	TAG
C16:0	62.9	69.0	77.0	62.8	73.2	65.5
C18:0	69.6	75.2	81.5	71.0	78.2	72.5
C18:1	13.0	23.5	35.2	–	25.8	4.9
C18:2	–5.2	8.9	12.3	–	–2.6	–13.1

[†] Figures are obtained from Lipid Bank for web (lipid.bio.m.u-tokyo.ac.jp).



X and Y are two types of fatty acids.

Fig. 18. Reaction scheme of enzymatic ester-ester exchange between two triacylglycerols (XXX and YYY) with *sn*-1,3 specific lipases.

method for the modification of oils and fats. The reaction is given in Fig. 18.

Enzymatic interesterification is different, in principle, from the chemical one in the case that the lipase used is *sn*-1,3 specific. It is not a random reaction in this case as shown in Fig. 18. The *sn*-1,3 positions will be exchanged and the *sn*-2 position will be maintained. In chemical interesterification, all positions will be randomized even though 8 triacylglycerols are still formed as shown in Fig. 18, but the contents of each TAG will be different, depending on how the fatty acids are located in *sn*-1,3 positions and *sn*-2 po-

sition. Since the DAGs are formed as intermediates, acyl migration is also taking place during the reaction. This will lead to randomization if the reaction time is not limited. Suppose that the reaction is randomized, the TAG profile of the interesterified product can be calculated from its fatty acid composition. The equations for the calculation of different types of TAG species are listed below:

$$[\text{AAA}] (\text{mol-}\%) = a^3/10^4$$

$$[\text{ABB}] (\text{mol-}\%) = 3ab^2/10^4$$

$$[\text{ABC}] (\text{mol-}\%) = 6abc/10^4$$

where a, b, c are the fatty acid content (mol-%) of A, B, C, respectively. Comparing to the blended mixture, the TAG profiles of randomized and enzymatically interesterified products differ a lot. The two interesterified products are slightly different even though they are very close in TAG profiles (Fig. 19).

In Fig. 18, the reaction is reversible and the equilibrium should be reached at the end. The reaction is different from other reactions discussed above such as hydrolysis, esterification, acidolysis, or alcoholysis. All other reactions often lead to the formation of “side-products” such as glycerol, water, released fatty acids, and released al-

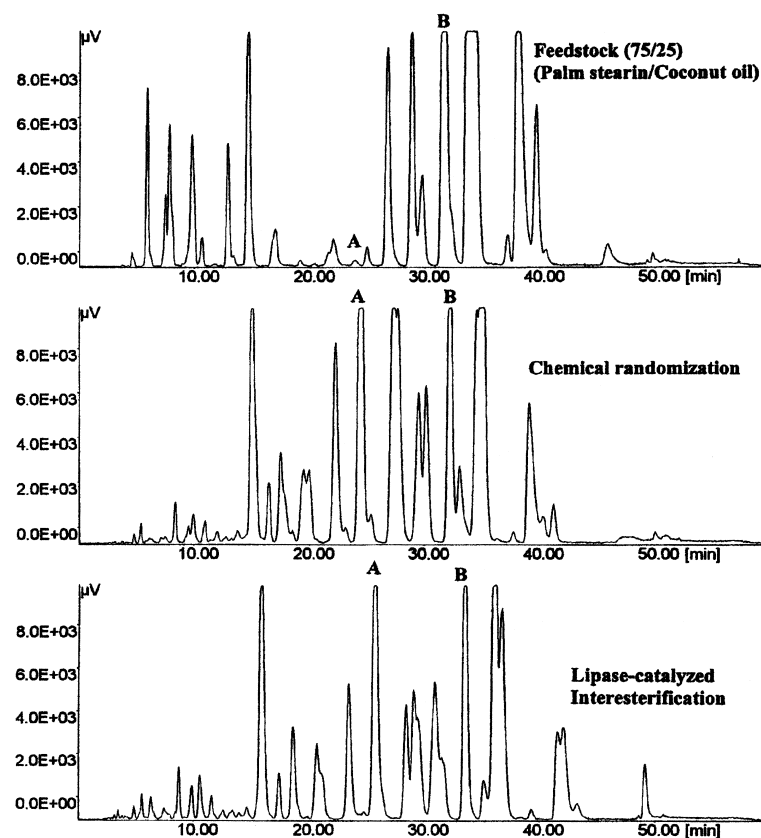


Fig. 19. A comparison between HPLC chromatograms of the palm stearin and coconut oil blend (feedstock), and products from chemical randomization and lipase-catalyzed interesterification. Peaks A and B are particularly different and can be used as markers.

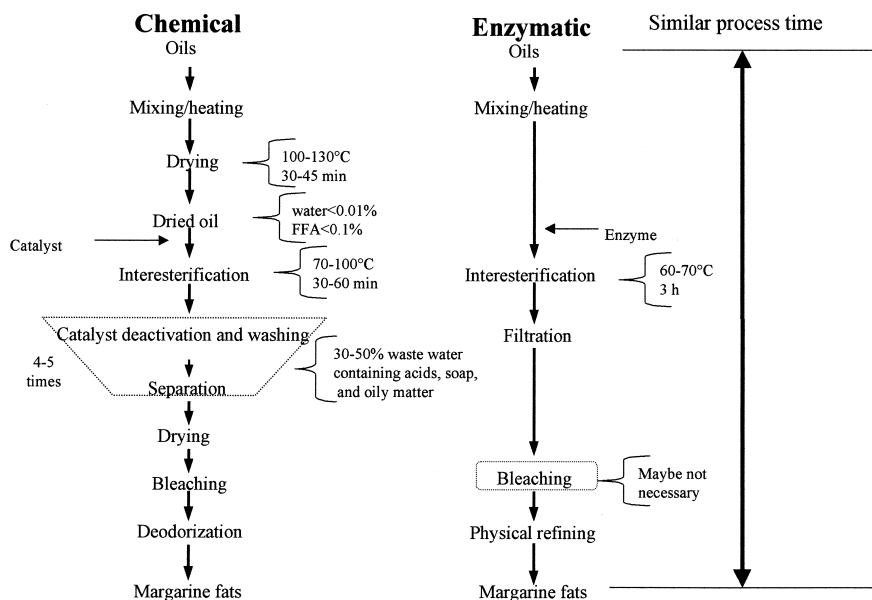


Fig. 20. A process comparison between chemical and enzymatic ester-ester exchange in stirred tank reactors.

cohol, respectively. These products can be removed, since they have other properties than the reaction substrates. This operation can often lead to higher product yields by breaking the reaction equilibrium and driving the reaction towards the product side. In the TAG-TAG reaction in Fig. 18 from the left to the right side, the consideration is different from the points depicted above and the purpose is to minimize the difference between “products” and the starting oils and fats. Therefore it is not so easy to apply any special operation to drive the reaction towards the right side by breaking the reaction equilibrium.

On the other hand the reaction can be driven to the left side to form solid fat and liquid oil fractions by reducing the reaction temperature to a certain low level depending on the starting oils and fats. During the reaction catalyzed by a lipase, the solid fraction, XXX for example, will be solidified to move out of the system. This leads to the formation of more XXX through the ester-ester exchange among different TAG species. The reaction can lead to the final formation of two main fractions, a solid fraction (XXX) and a liquid fraction (YYY for example). This operation is named “directed interesterification” and has been used for the chemical modification of oils and fats as well.

As applied in the chemical modification of oils and fats, the major application of ester-ester exchange has been focused on the production of margarine fats, which have a big and strong market. Margarine fats are daily-used but usually low-priced products. The uses of enzyme technology will meet further challenge with respect to economical balance of the costs and turnovers, comparing to the applications for those higher-valued products. The benefits and shortcomings of the technology should be clearly ex-

plored and weighted against each other. Among them, process and side reactions, which are the topics of this paper, are two aspects that should be concerned to make the enzyme method a benefits-weighted technology. Enzyme processes are simpler in general than chemical interesterification. A simple comparison between chemical and enzymatic processes using batch STRs is given in Fig. 20. For continuous operations, enzyme process is even simpler in which a PBR can be used as shown in Fig. 14. The product or the effluent can then be pumped to the deodorizer for physical refining.

Side reactions of hydrolysis in the enzyme process are a companion since enzymes, in general, require a certain amount of water in the system. This often leads to the formation of FFA and DAGs into the final products. The amount of water required for the active reaction depends on the type of lipases. Lipozyme TL IM for example is less affected by the amount of water and therefore the amount of water in the system can be reduced to a very low level [30]. The formation of FFA can thus be minimized. In general it is believed that water affects the rate of reaction. The reaction can be described as the hydrolysis-esterification mechanism. For the hydrolysis step, water is actually required acting as a reactant. The formed DAG can esterify with another FFA to form a new TAG. DAGs are therefore the intermediates to allow the exchange of acyls between hydroxyl groups (-OH) in the glycerol backbone. The rate of DAG formation is related to the water content in the system. However, water in the system will result in the formation of FFA and DAGs at the end. For the consideration of both, a high reaction rate and a low FFA content, the latter period of the reaction can be conducted at a reduced water

content through the application of vacuum. This will lead to more esterification and less hydrolysis.

Ester-ester exchange can also be used for the production of structured TAGs with the reaction between TAGs and fatty acid ethyl esters. The reaction scheme is actually similar to the one of acidolysis, and regio-incorporation of particular fatty acids into *sn*-1,3 positions can be accomplished with specific lipases. This is promising especially for those lipases being inhibited by free fatty acids. The reaction and process engineering is very close to that for acidolysis.

7 Reaction and reactor optimization

Optimization is the way of getting the best out of a given system. In most aspects of industrial applications of enzymatic modifications, continual improvement is an important feature. This is required both for quality improvement of the products and for highest economical turnover. Optimization is a formal presentation of these ideas. Optimization is usually an engineering operation that is involved in physical and/or chemical modeling and mathematical manipulation. However, in a real reaction system, complete theoretical models predicting the truth of the reaction or the reactor are not always available. In many circumstances, the attempt to develop a model expressing physical, chemical, or thermodynamical laws could be of great difficulty and probably unjustified. In all these situations, the practical optimization of a real system can be made with mathematical tools such as the commonly used response surface methodology (RSM).

RSM is an experimental strategy consisting of a particular set of mathematical and statistical methods. Many commercial programs have been developed and can be used to assist the optimization of a system. This has made optimization easier and accessible to common researchers who may not be a mathematician.

Optimization using RSM is very useful for enzymatic modification systems. One reason is that there are no true theoretical models able to fully predict such a complex system including the effects of enzyme characteristics, mass and heat transfer, factors, stability, water thermodynamics, etc. Another reason is that several factors have interactions and jointly and simultaneously influence the desired responses. A satisfactory RSM optimization can provide a lot of valuable information for the applied system. (i) Optimization can provide a statistical mathematical model between responses and factors. Usually quadratic polynomial models are used to approximate the responses. After the response from experiments is fitted, a mathematical relationship is set up between the response and all factors. The model obtained can therefore be used for

any mathematical manipulations like any other theoretical models, for example, to predict responses at any given variable conditions in the defined ranges. (ii) Response surfaces and contour plots can be generated to characterize the shape of the surfaces and locate the optimum. (iii) An evaluation of the significance of factors can be performed. The main effect of each factor on the response can also be evaluated.

The shortcoming of using RSM for experimental optimization should be always kept in mind. Since the model is derived from the defined ranges of each factor, extrapolation is impossible. The model can only be used in the defined ranges for optimization or prediction [31].

8 Product development

Enzymatic lipid modification and synthesis for product development can be generally applied in the following two categories:

- Process development of conventionally available products with improved quality
- Product and process development of new products, especially products impossible to be produced by chemical or traditional methods

In principle, many chemical methods can be replaced by enzymatic ones. Therefore lipid products that can be produced by enzymatic routes are numerous and difficult to be summarized here. For acylglycerols associated with fatty acids, many different products can be produced through enzymatic re-structuring. The possibilities are depicted in Fig. 21. In the first row, alkyl esters can be produced *via* alcoholysis of oils and fats. Fatty acid fractionation is suitable for the enrichment of particular fatty acids by using the selectivity of lipases toward different fatty acids. Particular applications for the enrichment of PUFA such as EPA and DHA, GLA (gamma-linolenic acid), and CLA (conjugated linoleic acid) have been studied. Different reactions have been used to achieve the targets and

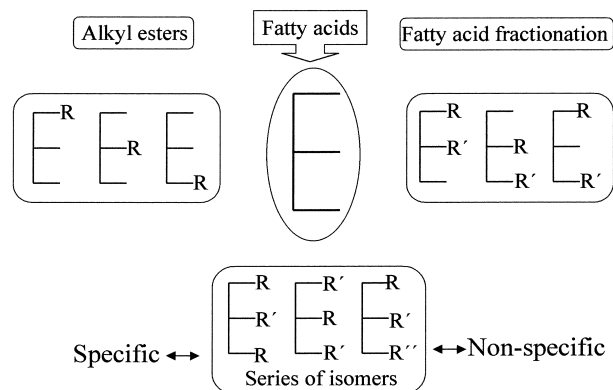


Fig. 21. Routes to re-structure acylglycerols.

efficiency, such as hydrolysis, esterification, alcoholysis, etc. [32–33].

Partial acylglycerols are located in the second row of Fig. 21. MAGs are traditional lipid products used as food emulsifiers or surfactants in other applications. DAGs are also intensively discussed for the reduction of fat accumulation in human body. Both MAGs and DAGs are the intermediates between TAGs and glycerol/FFA, which can be synthesized by different reaction routes [24–25]. A summary of these routes is depicted in Fig. 22. As shown in the figure, DAGs and MAGs are part of the reaction equilibrium. The only way to generate more products is to break the equilibrium and force the equilibrium to the formation of these products. This has been discussed in the early sections.

Structured TAGs are located in the bottom row of Fig. 21. For those specific structured TAGs, enzymatic methods are only feasible ways for large-scale productions. This has been the major focus of attention for decades due to the following considerations:

- products are valuable and high-priced referring to nutritional and functional properties,
- chemical methods are impossible for wide uses for the production of food related ingredients,
- market demands of related products are in high concern,
- economical balance can be maintained even with high cost of lipases.

There are a number of products available with a specific structure, which have been developed in the last 20 years. Some of them have been commercialized. The typical products are depicted in Fig. 23 for their targeted structure. The production of these structured TAGs mainly involves the acidolysis reaction or the TAG-ethyl ester exchange reaction in order to exploit the regiospecificity of lipases [5]. Steps of other reactions can also lead to the formation of specifically structured TAGs [27–28]. This has been discussed in the above sections. For the non-spe-

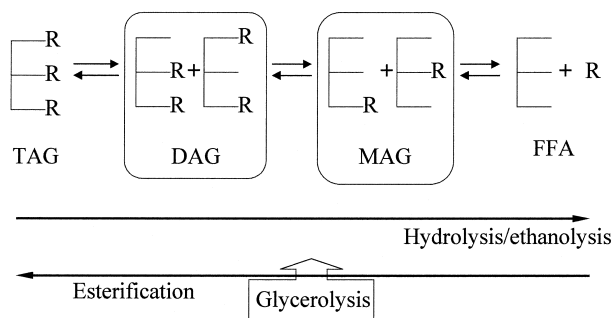


Fig. 22. Diacylglycerol (DAG) and monoacylglycerol (MAG) are intermediates of all the reaction methods including hydrolysis, alcoholysis, esterification, and glycerolysis in the equilibrium systems.

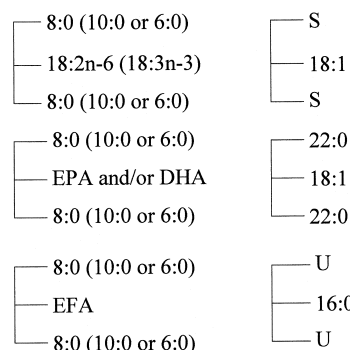


Fig. 23. Typical specific structured triacylglycerols. EFA – essential fatty acid, S – saturated fatty acids, U – unsaturated fatty acids.

cific TAG products, the research has been focused on margarine fats, which nearly exclusively involves the ester-ester exchange reaction.

9 Process technology

Enzyme reactors are central part of enzyme processes. Many different reactors have been studied and used for the enzymatic modification of lipids, such as STRs, PBRs, membrane reactors, gas-lifting reactors, fluidized reactors, simple circulating reactors, spraying reactors, foam reactors, etc. with/without internal or external extractive or separation steps or other enforcing steps [34–37]. This list is certainly not a scientific classification. Some of those typical reactors or process configurations or their combinations have been discussed in some of the above sections.

There are no universal criteria for the choice of a reactor. The following can be used for the evaluation of a suitable reactor for a particular system and process:

- reaction efficiency (homogeneity, dispersion, enzyme activity, etc.),
- product quality (yield and purity),
- enzyme reuses or stability,
- process feasibility and practicality,
- process productivity,
- process investment and availability,
- process and product change flexibility,
- easiness of process operation, control, and monitoring.

The influence of each above-mentioned criterion can be arbitrary from case to case, however in general, factors are listed by decreasing significance from scientific point of view. An efficient reaction is the most essential requirement for a particular system.

PBRs are one of the most interesting reactor types that have currently received a lot of attention and applications in the lipid modification industry. The particular concerns for the application of PBRs in lipid modification can be:

- simple process and operation,
- no filtration of enzyme necessary,
- less damage for the enzyme particle size and structure,
- continuous process easy and enzyme reuses simple,
- low investment,
- less reaction time and in turn leading to less side reactions in those specific reactions (better quality),
- efficient reaction under optimal operation.

A few things on the other hand should be carefully controlled or avoided, such as:

- internal and external mass transfer limitations,
- channeling under either upward or downward flow,
- pressure drops under smaller size of enzyme particle or higher flow rate,
- water content control and regulation,
- fouling of enzyme bed during the process running.

All these issues require a thorough understanding of the process and system. In general the design of the unit PBR should limit to a certain capacity in order to achieve above issues under optimal operations. In general a smaller size of the unit PBR has many advantages such as: Easy to change, low pressure drop, easy to avoid channeling, low cost for optimization, easy to control mass/heat transfer, easy to maintain a stable productivity, easy to maintain a suitable linear flow rate, easy to expand the capacity by parallel connection of a few PBRs.

Considering the reduction of enzyme activity, flow rates should be constantly adjusted and reduced to obtain a stable product quality and yield in a single PBR system. This is often not practical for the implementation of the technology in the industrial plants since stable process capacity cannot be maintained, even though the automatic adjustment of flow rates can be conducted in the present electronic modern world. The way to maintain a stable production capacity for a practical process is to make a series PBR design in which a number of unit PBRs are set in series along the flow line and the ages of the enzyme in each PBR are different (Fig. 24). The used-up PBR can be moved out of the series from the first step and the new one is then inserted into the series in the last step. In this way a stable production capacity can be maintained with little fluctuation if the number of unit PBRs is sufficient. In a PBR system for the

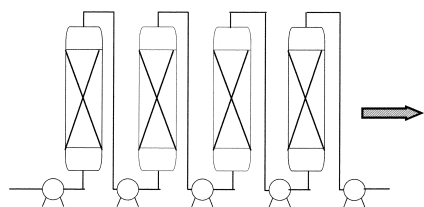


Fig. 24. Series setup of packed bed reactors.

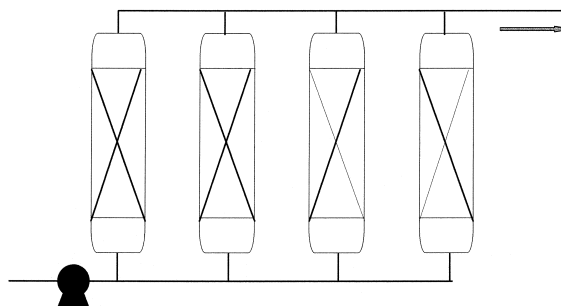


Fig. 25. Parallel setup of packed bed reactors.

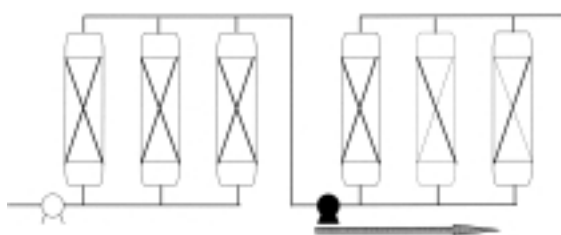


Fig. 26. Series and parallel setup of packed bed reactors.

production of structured lipids from sunflower oil and caprylic acid with the reaction of acidolysis, four columns in a series resulted in a 2–3 mol-% fluctuation from the average incorporation of caprylic acid into sunflower oil (47 mol-%) compared to the two columns in series with 4–5 mol-% fluctuation from the average (43 mol-%). In principle, seven columns in series can lead to a production yield or capacity with the fluctuation less than 5% with whatever the enzyme activity and stability.

With reference to the benefits of using a smaller size PBR, process capacity can be enlarged by parallel connections of more than one PBR (Fig. 25). Considering the necessity of series connection setup for a stable production capacity, parallel and series connections can be implemented in the same process setup (Fig. 26). By this operation, the process can provide the required flexibilities with a stable and practical production.

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References

- [1] A. Zaks, A. M. Klivanov: Enzymatic catalysis in organic media at 100 °C. *Science* **224** (1984) 1249–1251.
- [2] Engineering of/with Lipases. Ed. F. X. Malcata, Kluwer Academic Publishers, Dordrecht (The Netherlands) 1996.
- [3] U. T. Bornscheuer, R. J. Kazlauskas: Hydrolases in organic synthesis: Regio- and stereo-selective biotransformation. Wiley-VCH, Weinheim (Germany) 1999.
- [4] U. Bornscheuer (Ed.): Enzymes in Lipid Modification. Wiley-VCH Weinheim (Germany) 2000.
- [5] X. Xu: Production of specific-structured triacylglycerols by lipase-catalyzed reactions: A review. *Eur. J. Lipid Sci. Technol.* **102** (2000) 287–303.
- [6] T. M. Kuo, H. W. Gardner (Eds.): Lipid Biotechnology. Marcel Dekker, New York (USA) 2002.
- [7] S. H. Krishna, N. G. Karanth: Lipases and lipase-catalyzed esterification reactions in nonaqueous media. *Catal. Rev.* **44** (2002) 499–591.
- [8] K. Schroen: Ph.D. Thesis, Wageningen Agricultural University, 1995.
- [9] K. E. Rice, J. Watkins, C. G. Hill: Hydrolysis of menhaden oil by a *Candida cylindracea* lipase immobilized in a hollow-fiber reactor. *Biotechnol. Bioeng.* **63** (1999) 33–45.
- [10] F. Shahidi, U. N. Wanasundara: Omega-3 fatty acid concentrates: Nutritional aspects and production technologies. *Trends Food Sci. Technol.* **9** (1998) 230–240.
- [11] A. R. Medina, E. M. Grima, A. G. Gimenez, M. J. I. Gonzalez: Downstream processing of algal polyunsaturated fatty acids. *Biotechnol. Adv.* **16** (1998) 517–580.
- [12] A. R. M. Yahya, W. A. Anderson, M. Moo-Young: Ester synthesis in lipase-catalyzed reactions. *Enzyme Microb. Technol.* **23** (1998) 438–450.
- [13] N. N. Gandhi: Applications of lipase. *J. Am. Oil Chem. Soc.* **74** (1997) 621–634.
- [14] L. Q. Cao, A. Fischer, U. T. Bornscheuer, R. D. Schmid: Lipase-catalyzed solid phase synthesis of sugar fatty acid esters. *Biocatal. Biotrans.* **14** (1997) 269–283.
- [15] J. A. Arcos, H. S. Garcia, C. G. Hill: Continuous enzymatic esterification of glycerol with (poly)unsaturated fatty acids in a packed-bed reactor. *Biotechnol. Bioeng.* **8** (2000) 563–570.
- [16] Y. Isono, M. Nakajima, H. Nabetani: Solvent-free esterification of oleic acid and oleyl alcohol using membrane reactor and lipase-surfactant complex. *J. Ferment. Bioeng.* **86** (1998) 138–140.
- [17] K. Belafi-Bako, N. Dormo, O. Ulbert, L. Gubicza: Application of pervaporation for removal of water produced during enzymatic esterification in ionic liquids. *Desalination* **149** (2000) 267–268.
- [18] X. Xu, H. Mu, A. R. H. Skands, C.-E. Høy, J. Adler-Nissen: Parameters affecting diacylglycerol formation during the production of specific-structured lipids by lipase-catalyzed interesterification. *J. Am. Oil Chem. Soc.* **76** (1999) 175–181.
- [19] X. Xu, S. Balchen, C.-E. Høy, J. Adler-Nissen: Pilot batch production of specific-structured lipids by lipase-catalyzed interesterification: preliminary study on incorporation and acyl migration. *J. Am. Oil Chem. Soc.* **75** (1998) 301–308.
- [20] X. Xu, A. Skands, G. Jonsson, J. Adler-Nissen: Production of structured lipids by lipase-catalyzed interesterification in an ultrafiltration membrane reactor. *Biotechnol. Lett.* **22** (2000) 1667–1671.
- [21] X. Xu, S. Balchen, G. Jonsson, J. Adler-Nissen: Production of structured lipids by lipase-catalyzed interesterification in a flat membrane reactor. *J. Am. Oil Chem. Soc.* **77** (2000) 1035–1041.
- [22] X. Xu, S. Balchen, C.-E. Høy, J. Adler-Nissen: Production of specific-structured lipids by enzymatic interesterification in a pilot continuous enzyme bed reactor. *J. Am. Oil Chem. Soc.* **75** (1998) 1573–1579.
- [23] B. C. Paez, A. R. Medina, F. C. Rubio, P. G. Moreno, E. M. Grima: Production of structured triglycerides rich in *n*-3 polyunsaturated fatty acids by the acidolysis of cod liver oil and caprylic acid in a packed-bed reactor: equilibrium and kinetics. *Chem. Eng. Sci.* **57** (2002) 1237–1249.
- [24] U. T. Bornscheuer: Lipase-catalyzed syntheses of monoacylglycerols. *Enzyme Microb. Technol.* **17** (1995) 578–586.
- [25] L. Peng, X. Xu, T. Tan: Enzymatic production of high quality monoacylglycerols. In: *Research Advances in Oil Chemistry*. Ed. R. M. Mohan, GRN, Kerala (India) 2000, pp. 53–78.
- [26] X. Xu: Modification of oils and fats by lipase-catalyzed interesterification: aspects of process engineering. In: *Enzymes in Lipid Modification*. Ed. U. Bornscheuer, Wiley-VCH, Weinheim (Germany) 2000, pp. 206–231.
- [27] U. Schmid, U. T. Bornscheuer, M. M. Soumanou, G. P. McNeill, R. D. Schmid: Optimization of the reaction conditions in the lipase-catalyzed synthesis of structured triglycerides. *J. Am. Oil Chem. Soc.* **75** (1998) 1527–1531.
- [28] R. Irimescu, K. Furihata, K. Hata, Y. Iwasaki, T. Yamane: Utilization of reaction medium-dependent regiospecificity of *Candida antarctica* lipase (Novozym 435) for the synthesis of 1,3-dicapryloyl-2-docosahexaenoyl (or eicosapentaenoyl) glycerol. *J. Am. Oil Chem. Soc.* **78** (2001) 285–289.
- [29] Y. Shimada, Y. Watanabe, T. Samukawa, A. Sugihara, H. Noda, H. Fukuda, Y. Tominaga: Conversion of vegetable oil to biodiesel using immobilized *Candida antarctica* lipase. *J. Am. Oil Chem. Soc.* **76** (1999) 789–793.
- [30] H. Zhang, X. Xu, J. Nilsson, H. Mu, C.-E. Høy, J. Adler-Nissen: Production of margarine fats by lipase-catalyzed interesterification with a new immobilized *Thermomyces lanuginosa* lipase- a large-scale study. *J. Am. Oil Chem. Soc.* **78** (2001) 57–64.
- [31] X. Xu: Response surface methodology for experimental optimization. *Lipid Technol.* **1** (2002) 17–19.
- [32] U. N. Wanasundara, F. Shahidi: Lipase-catalyzed concentration of *n*-3 polyunsaturated fatty acids in acylglycerols from marine oils. *J. Am. Oil Chem. Soc.* **75** (1998) 945–951.
- [33] G. G. Haraldsson, B. Kristinsson: Separation of eicosapentaenoic acid and docosahexaenoic acid in fish oil by kinetic resolution using lipase. *J. Am. Oil Chem. Soc.* **75** (1998) 1551–1556.
- [34] X. Xu: Enzyme bioreactors for lipid modifications. *INFORM* **11** (2000) 1004–1012.
- [35] V. M. Balcao, A. L. Paiva, F. X. Malcata: Bioreactors with immobilized lipases: state of the art. *Enzyme Microb. Technol.* **18** (1996) 392–416.
- [36] D. M. F. Prazeres, J. M. S. Cabral: Enzymatic membrane bioreactors and their applications. *Enzyme Microb. Technol.* **16** (1994) 738–751.
- [37] A. L. Paiva, V. B. Balcao, X. Malcata: Kinetics and mechanisms reactions catalyzed by immobilized lipases. *Enzyme Microb. Technol.* **27** (2000) 187–204.